the lactate upon acid hydrolysis. Toxic components included 7.5 percent cacodylate and 0.5 percent methanearsonate as determined by radiochromatographic analysis of kidney extracts of ^{74}As-labeled animals. The ratios of these compounds are similar to those produced from [^{74}As]arsenate by isolated zooxanthellae of the *Tridacna* mantle. As with CO_2 fixation products (*9*), such algae released part of their arsenic compounds to the surrounding seawater medium (7 percent cacodylate, 21 percent trimethylarsoniumlactate, and 72 percent of its unidentified derivative).

Concentric concretions occur in the kidneys of *Tridacna* as well as in the kidneys of other mollusks (*10*); their remarkable heavy metal contents have been reported. Using radioarsenic, we found that these concretions can serve as absorbents for [^{74}As]arsenate and [^{74}As]arsenite, much as claimed for similarly concentric concretions (*11*), the Bezoar stones of early pharmacy (*12*). Reports of arsenic detoxication by Bezoar stone have been published (*13*).

The accumulation of algal arsenicals in the clam kidney suggests that their excretion by way of the urine is slow. The gills appear to be a major avenue for arsenic release to the sea. *Tridacna maxima*, maintained for 24 hours in radioarsenate, incorporated equal amounts of radioactivity into the kidney and the gills (the arsenic content of gill tissue was only 5.2 ppm). The gill radioactivity was distributed among at least four novel membrane arsenolipids. Gill membranes exposed to bacterial oxidative activity, therefore, provide a site for arsenic excretion in *Tridacna* and possibly for other organisms as well.

Arsenate is absorbed and methylated primarily by algae growing in tropical waters where phosphate concentrations may be comparably low. This observation is reflected in the generally low arsenic concentrations measured for temperate-water mollusks (Table 1) and reported for marine fishes and invertebrates (*1*, *14*) from northern waters of much higher phosphate concentrations. The results demand further study of the regulation of arsenate metabolism by environmental phosphate and a comparative study of renal excretion of known arsenical metabolites.

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Phenytoin-Induced Teratogenesis: A Mouse Model

Abstract. *Malformations associated with the fetal hydantoin syndrome have been reproduced in a mouse model. The occurrence of these defects was correlated with maternal serum concentrations, but not with maternal or fetal genotype or the presence of a seizure disorder.*

The suspected teratogenicity of phenytoin (Dilantin; Parke, Davis) in humans requires investigation, especially since 0.3 to 0.5 percent of pregnant women are epileptic and therefore candidates for anticonvulsant drug therapy (*1*). The association between epilepsy, birth defects, and anticonvulsant drugs was recognized in 1963 (*2*), but recently clinical reports have indicated a specific pattern of abnormalities in the offspring of mothers receiving hydantoin anticonvulsants during pregnancy (*3–7*). This pattern, known as the fetal hydantoin syndrome, includes craniofacial malformations, developmental and psychomotor delay, pre- and postnatal growth deficiency, impaired intellectual performance, and cardiac, genitourinary, and skeletal anomalies (*6*).

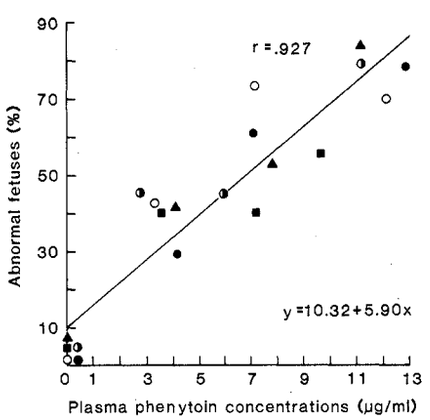

Fig. 1. Relation between the maternal plasma concentration of phenytoin and the frequency of fetuses with malformations. Data points represent averages of all litters: (○) C57 (+/+); (◑) C57 (+/*qk*); (●) C57 (*qk*/*qk*); (■) C_3H; and (▲) SWV.

It has been argued that maternal anticonvulsant therapy is but one variable common to the syndrome (*8*). Shapiro *et al.* (*9*), analyzing data from a collaborative perinatal project, concluded that epilepsy, either maternal or paternal, is the decisive factor in the etiology of the malformations, and not a teratogenic effect of the anticonvulsant drugs. Rather than trying to factor out all of the possible etiologic variables in an epidemiologic approach to the problem, we developed an animal model of the fetal hydantoin syndrome.

To separate the teratogenic effect of epilepsy from that of phenytoin treatment, we required an animal model that would closely approximate the human condition by meeting the following criteria (*10*): (i) The test animal should have spontaneous seizures. (ii) The seizures should be controlled or eliminated by phenytoin treatment. (iii) The drug should be administered orally. (iv) Plasma drug concentrations should fall within the optimal therapeutic range for mice, of 2.5 to 10 μg per milliliter of plasma. (v) The offspring of treated ani-

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mals should exhibit the spectrum of malformations observed in the offspring of epileptic women.

Three inbred mouse strains, SWV, C_3H and C57BL/6J, were used in this study. The autosomal recessive gene quaking (*qk*) on the C57BL/6J background, which confers a spontaneous tonic-clonic seizure disorder on homozygous (*qk/qk*) mice (*11*) was an integral part of this experiment. To separate the role of the quaking gene from the effects of phenytoin on the etiology of the malformations, phenotypically normal heterozygous (+/*qk*) and homozygous nonquaking (+/+) dams were used as congenic control groups. The animals received the drug in drinking water for 2 weeks before the first attempts at mating, so that drug-metabolizing enzyme systems would be fully activated, and this therapy was continued throughout gestation. The females were mated to drug-naïve males of their respective strains. The fetuses were removed on day 18 of gestation and processed with standard teratological techniques (*10*).

Phenytoin was not excessively lethal to embryos, there being no statistically significant increases in the frequency of resorbed fetuses as the dosage level increased for any of the strains except the heterozygous (+/*qk*) C57BL/6J genotype (Table 1). The mean differences between measurements were tested with one-way analysis of variance. When differences were found to be significant at $P < .05$, a Student-Newman-Keuls range test was performed to discern the source of the significance (*12*). Fetal weights decreased significantly in all strains as the dosage of phenytoin increased. The incidence of animals born with one or more malformations increased dramatically with drug dosage and therefore with phenytoin concentrations in the maternal plasma. This was true for all strains and genotypes. The (*qk/qk*) females whose seizure disorder was left untreated produced normal offspring, even when seizures occurred daily throughout gestation—an indication that the drug, not the presence of a maternal seizure disorder, is the agent responsible for the malformations.

Although some malformations associated with the human fetal hydantoin syndrome were reported in earlier animal studies (*13–18*) we have now reproduced the complete syndrome. Among the anomalous systems in both human and mouse fetal hydantoin syndromes are growth deficiency, evidenced by low fetal weights and incomplete ossification, and ocular, neural, cardiac, renal, and skeletal defects (*10*). The malformations we observed most often were dilated or immaturely developed cerebral ventricles, ventricular septal defects, hydronephrotic kidneys, and digital hypoplasia. Fetuses were removed from the dams prior to parturition, and perinatal death was not directly observed but was indicated by the high frequency of cardiac and renal defects.

With the aid of an animal model of the fetal hydantoin syndrome that closely parallels the human disorder, we have demonstrated that the anticonvulsant drug phenytoin, not the presence of a maternal seizure disorder, is responsible for the increased incidence of congenital defects. The risk of producing offspring with a malformation is directly correlated with the maternal plasma phenytoin concentration (Fig. 1). There was no evidence of drug interaction with either the maternal or fetal genotypes.

Because the reported plasma phenytoin concentrations are teratogenic within the therapeutic range, it is strongly suggested that this and similar compounds be avoided whenever possible in women of reproductive age who are contemplating pregnancy.

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Table 1. Effect of phenytoin treatment on implants, resorptions, live births, fetal weights, abnormalities, and maternal plasma phenytoin concentrations. Fetal weights and plasma phenytoin concentrations are given as means ± standard errors. Female mice of all strains were randomly assigned to one of four treatment groups (0, 20, 40, and 60 mg of phenytoin per kilogram of body weight). Phenytoin was administered in the animals' drinking water, the *p*H of which had been raised to 10.2 by the addition of 2*M* NaOH. Plasma phenytoin concentrations were determined from samples obtained from the retroorbital sinus of dams on days 5, 10, and 15 after the initiation of drug therapy, and also at the time of pregnancy termination, using a modification of the high-pressure liquid chromatographic technique of Kaba *et al.* (*19*).

Strain and genotype	Phenytoin treatment (mg/kg)	Implants (No.)	Live births (No.)	Resorptions (%)	Fetal weight (g)	Abnormalities (%)	Plasma phenytoin concentration (μg/ml)
SWV*	0	121	108	11	0.81 ± 0.01	6	0
	20	119	101	15	0.73 ± 0.01	41	4.13 ± 0.51
	40	118	97	18	0.68 ± 0.02	53	7.77 ± 0.58
	60	115	99	14	0.64 ± 0.01	85	11.03 ± 1.48
C_3H*	0	102	98	4	0.99 ± 0.01	4	0
	20	102	89	13	0.86 ± 0.01	40	3.49 ± 0.42
	40	92	75	20	0.84 ± 0.01	40	7.13 ± 0.55
	60	94	80	15	0.82 ± 0.01	56	9.54 ± 0.42
C57 (+/+)†	0	44	43	2	0.98 ± 0.02	2	0
	20	36	33	8	0.80 ± 0.02	42	3.22 ± 0.17
	40	39	33	15	0.69 ± 0.02	73	7.05 ± 0.81
	60	38	30	21	0.73 ± 0.02	70	12.10 ± 1.04
C57 (+/*qk*)†	0	44	41	7	1.03 ± 0.02	5	0
	20	40	39	2	0.77 ± 0.02	44	2.69 ± 0.23
	40	37	32	14	0.85 ± 0.03	44	5.77 ± 0.70
	60	44	32	25	0.72 ± 0.01	78	10.96 ± 0.90
C57 (*qk/qk*)†	0	39	36	8	1.05 ± 0.03	0	0
	20	37	34	8	0.85 ± 0.02	29	4.12 ± 0.54
	40	40	38	5	0.72 ± 0.02	61	7.40 ± 0.60
	60	32	26	19	0.75 ± 0.02	77	12.43 ± 1.90

*Ten litters for each treatment. †Five litters for each treatment.

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